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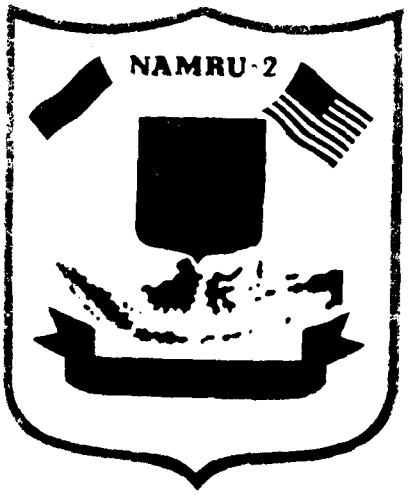

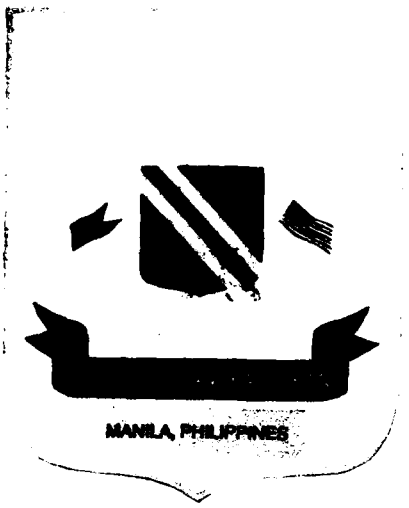

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Zika virus, a cause of fever in Central Java, Indonesia

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Summary

In 1977 and 1978 selected in-patients at the Tegalyoso Hospital, Klaten, Indonesia who had recent onsets of acute fever were serologically studied for evidence of alphavirus and flavivirus infections. A brief clinical history was taken and a check list of signs and symptoms was completed on admission. Acute and convalescent phase sera from 30 patients who showed evidence that a flavivirus had caused their illnesses were tested for neutralizing antibodies to several flaviviruses which occur in South-east Asia. Paired sera from seven patients demonstrated a fourfold rise in antibody titre from acute to convalescent phase. The most common clinical manifestations observed in this series of patients included high fever, malaise, stomach ache, dizziness and anorexia. None of the seven patients had headache or rash despite the fact that headache and rash had been associated with two of the three previously studied. The onsets of illness clustered toward the end of the rainy season when populations of *Aedes aegypti*, a probable vector in Malaysia, were most abundant.

Introduction

Zika virus (ZIKA) was first isolated from a sentinel Rhesus monkey in the Zika Forest of Uganda in 1947 (DICK *et al.*, 1952) and since has been recovered from the following African mosquito species: *Aedes africanus* collected in Uganda (DICK *et al.*, 1952; SMITHBURN, 1952 and HADDOW *et al.*, 1964) and in the Central African Republic (BERGE, 1975); from *Ae. luteocephalus* and *Ae.* spp. in Nigeria (LEE & MOORE, 1972). The virus has also been isolated in Malaysia from *Ae. aegypti* (MARCHETTE *et al.*, 1969).

Serological evidence that ZIKA infects man is widespread and includes neutralizing (Nt) antibodies in residents of Uganda (DICK *et al.*, 1952; SMITHBURN, 1952), Tanganyika (SMITHBURN, 1952), Nigeria (DICK, 1953), Egypt (SMITHBURN *et al.*, 1954), India (SMITHBURN *et al.*, 1954a), Malaysia (SMITHBURN, 1954; POND, 1963) the Philippines (HAMMON *et al.*, 1958), Thailand and Vietnam (POND, 1963) and Indonesia (Suharyono, Lubis, Nalim, Gubler and Ksiazek, unpublished data).

Description of the clinical syndrome associated with ZIKA infection is based on three single cases independently reported. The first patient was presumably infected by the bite of the mosquito vector (SIMPSON, 1964). Symptoms included headache and signs included fever and a generalized maculopapular rash. The duration of the illness was five days and the infection was considered mild. The second case was a laboratory infection (BERGE, 1975) and presented with a rash. The third was also a laboratory acquired infection (FILIPE *et al.*, 1973 and had rapid onset of fever, chills, sweating, retro-orbital headache, arthralgia and pains at the back of the neck but no rash or signs of respiratory involvement. Three additional isolations of ZIKA were made from febrile children in Ibadan, Nigeria, but no information about their clinical illnesses was recorded (MOORE *et al.*, 1975).

In this brief report we describe seven additional cases of illness probably caused by ZIKA and present evidence that ZIKA infection may be an important cause of fever in Central Java, Indonesia.

Materials and Methods

Study subjects were selected from in-patients at Tegalyoso Hospital, Klaten, Central Java by clinicians at the University of Gajah Mada who were investigating the importance of leptospiral infections as causes of fever in this population during 1977 and 1978. Criteria for patient selection included axial temperature of 38°C on examination and history of fever for three or more days on admission. A physician member of the team recorded the patient's age, sex and date of onset of symptoms and made an examination from which a check list of 34 signs and symptoms was compiled.

Acute and convalescent sera were collected and tested at the laboratories of the Jakarta Detachment of the U.S. Naval Medical Research Unit No. 2 (NAMRU-2) for evidence of antibodies to *Leptospira* sp. by the passive haemagglutination (PHA) test (SULZER *et al.*, 1975) and to alphaviruses and flaviviruses by haemagglutination inhibition (HI) tests (CLARK & CASALS, 1958) using sucrose-acetone extracted mouse brain antigens prepared from stock viruses obtained from the National Institute of Allergy and Infectious Diseases (NIAID). Paired sera which showed a fourfold increase in antibody titre from the acute to the convalescent phase were considered positive and those in which an antibody titre of 1:640 or greater was detected in either serum were presumptive of infection. The sera which showed evidence of infection with flaviviruses were sent by air to the NAMRU-2 Laboratory in Taipei, Taiwan where they were retested by micro-neutralization (KSIAZEK & YUILL, 1977) for antibodies to the following virus strains: Japanese encephalitis (JE), Nakayama; dengue type 2 (DEN-2), New Guinea C; ZIKA, MR 766; Tembusu (TMU), MM 1775 and Murray Valley encephalitis (MVE), original. Criterion for evidence that viruses were responsible for the illness was a fourfold increase in serum Nt antibody from acute to convalescent phase.

Table I—Serological results of paired sera tested by microneutralization

Serum Number		reciprocal of haemagglutination inhibiting antibody titre		reciprocal of neutralizing antibody titre				
		JE	DEN-2	JE	DEN-2	ZIKA	TMU	MVE
5751	A*	40	160	<10	10	40	<10	<10
	C	40	640	<10	10	80	<10	<10
5771	A	10	40	20	20	⩾160	<10	10
	C	40	80	<10	20	⩾160	<10	10
5783	A	<10	<10	<10	**<10**	**20**	<10	**<10**
	C	320	320	<10	**80**	**>160**	<10	**20**
5793	A	<10	<10	<10	**<10**	**10**	ND**	10
	C	20	40	<10	10	**40**	ND	<10
5795	A	320	2560	<10	40	80	ND	10
	C	640	640	<10	40	80	ND	10
5803	A	10	20	<10	<10	**10**	ND	<10
	C	40	320	<10	10	**40**	ND	<10
5807	A	40	80	<10	40	**20**	ND	10
	C	160	320	<10	20	**⩾160**	ND	10
5809	A	40	20	<10	**10**	10	ND	<10
	C	160	320	<10	**40**	40	ND	10
5821	A	20	40	<10	**40**	80	ND	<10
	C	640	1280	<10	**⩾160**	⩾160	ND	<10
5823	A	40	80	<10	**20**	⩾160	<10	<10
	C	2560	5120	<10	**160**	⩾160	<10	10
13164	A	160	80	10	40	**10**	10	20
	C	640	160	10	80	**160**	20	20
13168	A	80	80	<10	**40**	⩾160	10	<10
	C	5120	2560	10	**160**	⩾160	20	10
13180	A	80	80	<10	40	80	<10	10
	C	320	160	10	20	80	<10	10
13200	A	20	20	<10	**<10**	**40**	<10	10
	C	640	640	<10	**80**	**⩾160**	10	<10
13204	A	40	20	<10	**<10**	**<10**	<10	10
	C	640	320	<10	⩾160	**⩾160**	10	<10
13208	A	80	80	<10	**20**	**<10**	10	10
	C	5120	2560	<10	**⩾160**	**<40**	10	20
13214	A	<10	<10	20	**<10**	**<10**	10	<10
	C	160	40	10	**40**	**40**	20	10
13216	A	20	20	<10	**20**	**20**	10	<10
	C	5120	2560	<10	**⩾160**	**⩾160**	20	<10
13218	A	40	40	10	**<10**	⩾160	10	10
	C	1280	1280	10	**⩾160**	⩾160	20	20
13230	A	320	320	<10	80	⩾160	10	**<10**
	C	2560	2560	10	⩾160	⩾160	20	**20**

Table I—*Continued*—Serological results of paired sera tested by microneutralization

Serum Number		reciprocal of haemagglutination inhibiting antibody titre		reciprocal of neutralizing antibody titre				
		JE	DEN-2	JE	DEN-2	ZIKA	TMU	MVE
13236	A	80	40	<10	40	⩾160	<10	<10
	C	160	160	<10	80	40	10	10
13254	A	160	80	<10	**<10**	10	10	<10
	C	1280	640	<10	**⩾160**	10	<10	10
13256	A	610	40	10	20	**20**	10	10
	C	320	160	10	40	**⩾160**	<10	<10
13258	A	10	10	<10	**<10**	**<10**	<10	<10
	C	320	160	10	**40**	**80**	10	10
13268	A	320	80	**<10**	**<10**	**20**	**<10**	40
	C	2560	640	**40**	**⩾160**	**⩾160**	**20**	20
13270	A	40	40	<10	40	10	10	10
	C	320	160	<10	40	**⩾160**	10	<10
13272	A	<10	<10	<10	**<10**	**<10**	<10	<10
	C	160	160	<10	**20**	**20**	<10	<10
13282	A	160	80	<10	**20**	160	<10	<10
	C	320	320	<10	**160**	<10	10	10
13284	A	80	40	<10	**20**	40	<10	<10
	C	320	160	<10	**⩾160**	10	<10	<10
13286	A	20	20	<10	20	**20**	<10	10
	C	80	80	<10	20	**80**	10	10

	VIRUS				
	JE	DEN-2	ZIKA	TMU	MVE
HMAF					
JE	**160**	<10	<10	<10	<10
DEN-2	<10	**160**	<10	<10	<10
ZIKA	<10	<10	**⩾640**	<10	<10
TMU	<10	<10	<10	**⩾320**	<10
MVE	<10	<10	<10	<10	**320**
KUN	<10	<10	<10	<10	<10
SEP	<10	<10	<10	<10	<10
LGT	<10	<10	<10	<10	<10

HMAF — hyperimmune mouse ascitic fluid
KUN — Kunjin virus
SEP — Sepik virus
LGT — Langat virus

*A—acute phase antibody titre, C—convalescent phase antibody titre
**ND—Not done
***Fourfold rises in neutralizing antibody titre are indicated by **bold** type.

Table II—Clinical signs and symptoms recorded for patients with serological evidence of Zika virus infection

Patient No.	Age	Sex	Date of Onset	Symptoms
5793	16	F	31 May 77	high fever, malaise, chills, anorexia, vomiting, diarrhoea, stomach ache, dizziness and leg pain.
5803	14	M	2 June 77	high fever, arthralgia, myalgia, diarrhoea, dizziness, conjunctivitis, haematuria.
5807	12	M	1 June 77	high fever, malaise, constipation, stomach ache and dizziness.
13164	32	F	4 April 78	high fever, chills, stomach ache, dizziness and lymphadenopathy.
13256	12	F	5 June 78	high fever, malaise, anorexia, constipation, stomach ache, dizziness and hypotension.
13270	25	F	20 May 78	high fever, malaise, anorexia, diarrhoea and stomach ache.
13286	13	M	15 July 78	high fever, malaise, anorexia, constipation, stomach ache and hypotension.

*Diagnostic rise only in ZIKA neutralizing antibody titre from acute to convalescent phase sera was considered evidence of infection.

Results

Of the total of 219 patients' sera tested for alphavirus and flavivirus HI antibody, 108 (48%) were positive or presumptive. 60 (27%) showed evidence of flavivirus infections and 30 (14%) had evidence of alphavirus infection; 18 (8%) had rises in HI antibody to both alphaviruses and flaviviruses.

Microneutralization tests on 25 (83%) of 30 paired sera from patients who were positive by HI for infection with a flavivirus showed diagnostic rises in Nt antibody to one or more flaviviruses used in the test (Table I). Seven showed a diagnostic rise in ZIKA antibody titre only, seven in DEN-2 only, and one in MVE only. Eight showed diagnostic rises in both DEN-2 and ZIKA antibody and two patients had increases in DEN-2, ZIKA and another flavivirus. Tests of sera from five patients failed to show rises in Nt antibody titre to any flaviviruses used in test.

Table II lists the age, sex, date of onset and signs and symptoms recorded for each patient for whom ZIKA infection was serologically confirmed. All patients had high fevers on examination. Six of seven patients had stomach ache, five had malaise, five experienced dizziness and four were anorexic. Less frequently reported symptoms and signs were diarrhoea three, constipation three, hypotension two and chills two; arthralgia, myalgia, vomiting, conjunctivitis, haematuria, lymphadenopathy and leg pains were each noted in only one of the patients. None of the patients had a rash.

Discussion

In the seven patients whose paired sera showed diagnostic rises in ZIKA Nt antibody only, the cause of illness was thought to be ZIKA. An additional 10 patients (Table I) showed fourfold or greater increases in ZIKA antibody titre from acute to convalescent phase, but also showed diagnostic rises in antibody titre to DEN-2 and or other flaviviruses. We did not include these patients among those whose serological results more clearly implicated infection with ZIKA.

The clinical signs and symptoms exhibited by the patients who serologically responsed only to ZIKA were different from those reported previously by SIMPSON (1964) and BERGE (1975). Rash, which was the only sign noted in previous ZIKA infections documented by isolation of virus, was not seen in our series of patients. It is possible that rash was present and not noted, but each patient was checked by a physician for its presence on admission.

The clinical picture reported by FILIPE *et al.* (1973) of fever, chills, sweating, headache, arthralgia and neck pains but no rash fits more closely with the signs and symptons observed for the patients seen in our study. High fever, general malaise, stomach ache, dizziness and anorexia were the most commonly recorded symptoms.

The temporal distribution of the seven cases suggests that ZIKA infections occur towards the end of the rainy season. Heavy rainfall usually commences in November and continues through June in Central Java. The year 1977 was fairly typical for rainfall with nearly 200 mm of precipitation being recorded each month, January through June. During July through October almost no rain fell. The year 1978 was one of excessive rainfall. Each month, January through August received heavy rain-

fall ranging from 136 mm in May to more than 1000 mm in January.

Aedes aegypti, a possible vector in Malaysia (MARCHETTE *et al.*, 1969), has high population densities during the months when excessive rain occurs. This species transmits dengue viruses which result in increased evidence of dengue haemorrhagic fever in urban areas of Central Java and may be the vector of ZIKA. Also, *Ae. albopictus* was implicated in reported outbreaks of dengue fever in 1976 at a location near our study site (JUMALI *et al.*, 1979) and is known to be widely distributed throughout rural parts of Indonesia (SULIANTI SAROSO, 1978). This, considered with the isolation of ZIKA virus from a variety of other *Aedes* of the subgenus *Stegomyia*, may indicate that *Ae. albopictus* might also serve as a vector of the virus.

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In 1977 and 1978 selected in-patients at the Tegalyoso Hospital, Klaten, Indonesia who had recent onsets of acute fever were serologically studied for evidence of alphavirus and flavivirus infections. A brief clinical history was taken and a check list of signs and symptoms was completed on admission. Acute and convalescent phase sera from 30 patients who showed evidence that a flavivirus had caused their

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were tested for neutralizing antibodies to several flaviviruses which occur in South-east Asia. Paired sera from seven patients demonstrated a fourfold rise in antibody titre from acute to convalescent phase. The most common clinical manifestations observed in this series of patients included high fever, malaise, stomach ache, dizziness and anorexia. None of the seven patients had headache or rash despite the fact that headache and rash had been associated with two of the three previously studied. The onsets of illness clustered toward the end of the rainy season when populations of Aedes aegypti, a probable vector in Malaysia, were most abundant.